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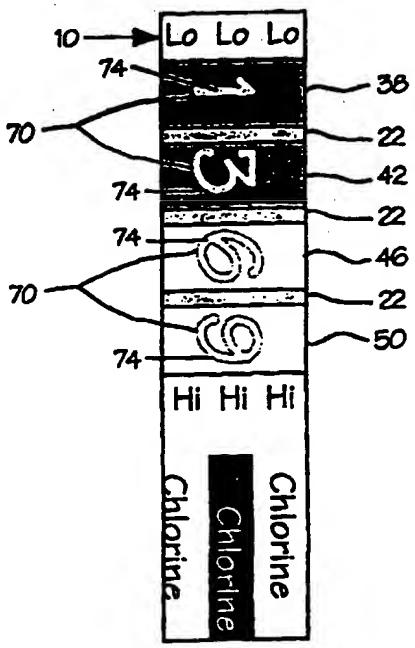
(54) Title: DIRECT READING CHEMICAL TEST STRIP FOR AQUEOUS SOLUTIONS

(57) Abstract

A chemical test strip comprised of a support layer, a first assay area, a second assay area and a hydrophobic zone positioned between the first assay area and the second assay area. The first and second assay areas are attached to the support layer and comprise an absorbent matrix. The first assay area includes a first test reagent absorbed in its matrix that changes color in response to a first concentration of an analyte such as free available chlorine. The second assay area includes a second test reagent absorbed in its matrix that changes color in response to a second concentration of the analyte. Interpretation of the test strip can be facilitated by printing information on the assay areas which is more visible after the color change than before the color change.



DIRECT READING CHEMICAL TEST STRIP FOR AQUEOUS SOLUTIONS

TECHNICAL FIELD

The present invention relates to a test strip for determining the concentration of analytes in aqueous solutions, and more particularly to a test strip having a plurality of test zones separated from each other by hydrophobic barriers.

BACKGROUND ART

The development of simple to use solid state photometric instruments has progressed dramatically since the first colorimetric tests for analytes were developed several decades ago. However, these instruments are still costly and may require periodic calibration and maintenance. Therefore, they tend to be used in only the most demanding, high volume applications.

In contrast, the basics of performing colorimetric tests without instrumentation have remained relatively constant. Visually interpreted colorimetric tests have been continuously utilized to provide an indication of the quantity of analyte in a solution. However, the problems associated with users performing a color match to a standard reference frequently result in errors.

The current nonautomated colorimetric testing devices are either aqueous titrations or test strips comprised of dry reagents impregnated on a bibulous (porous) matrix. The samples are either collected and placed in a vial for titration, or the dry reagent test strip is immersed in the sample and read. The tests are interpreted by visually matching the color change against a chart or set of standards. For example, Rupe et al., in U.S. Pat. No. 4,092,115, disclose a basic test strip which uses an indicator comprised of an azine compound to measure free available chlorine. The device described is an analog colorimetric test where the resulting indicator changes from yellow to violet.

A problem with color matching techniques is that the accuracy of such techniques is dependent on human perception of an analog scale, and is further complicated by environmental conditions such as lighting, fading of the standards and angle of comparison. One approach to reducing the problems

associated with color matching techniques is to design assay systems with results that are directly readable. For example, Hochstrasser, in U.S. Pat. No. 3,964,871, discloses a disposable indicator for measuring analytes in biological fluids which registers the concentration of the analyte with indicators (e.g. digital or other symbolic notation) that are directly readable. The test strip is used for detecting glucose in a biological sample and uses a plurality of chemistries having an indicator which undergoes a color change in the presence of hydrogen peroxide and peroxidase, and a compound which prevents the accumulation of oxidizer indicator until such time as said compound has been completely consumed in the reaction.

Another approach to simplifying the interpretation of test strips is to apply a scale directly to the test strip. For example, Ertinghausen, in U.S. Pat. 5,087,556 discloses a basic test strip which use an analog thermometer scale to provide a quantitative reading of a specified body fluid component. This test has at least one chromatic indicator immobilized in the membrane in predetermined concentrations.

SUMMARY OF THE PRESENT INVENTION

Briefly, the preferred embodiment of the present invention is a test strip for detecting and measuring the concentration of analytes in aqueous solutions. The types of analytes that can be detected include virtually any element, ion or compound, including chlorine, bromine, hydrogen ion (pH), hydroxyl ion (alkalinity), copper, lead, nitrate and the various substances, like calcium, that contribute to water hardness.

The test strip comprises a plurality of assay areas attached to an inert support layer, with each of the assay areas being isolated from each adjacent assay area by a hydrophobic barrier. Each of the assay areas is comprised of a bibulous (absorbent) matrix that has a test reagent, such as an indicator dye, absorbed in part of the void volume of the matrix. Each of the assay areas exhibits a "binary response" to the analyte meaning that the assay area remains one color until a threshold concentration of the analyte is exceeded. Once the threshold concentration of the analyte is exceeded, the test reagent causes the

assay area to change color. Each assay area has a specific reagent impregnated in the bibulous matrix which changes color at a different concentration of analyte. The results of the test are determined by identifying the number of assay areas that have converted as indicated by the color change. The test reagents are formulated to achieve the plurality of assay areas having these characteristics. Interpretation of the test strip may be facilitated by printing information on the assay areas, such as analyte concentration, which is more visible after the color change than before the color change.

The hydrophobic barriers provide a means of segregating and isolating the sample and chemicals applied to the assay areas so as to minimize cross contamination of samples and chemicals applied in each assay area. This segregation enables the performance of a series of titrations based on constant volume of sample size versus constant volume of indicating reagent. This volumetric relationship is based on the uniform absorption capability of the matrix. The indicating reagent is formulated so that the appropriate amount of indicator remains after the drying phase of manufacturing.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an isometric view of a test strip according to the present invention;

Fig. 2 is a partial cross-sectional view of the test strip taken along the line 2-2 in Fig. 1;

Fig. 3 is an isometric view of the test strip shown in Fig. 1 after development of the test strip;

Fig. 4 is a top view of an undeveloped test strip having information printed on the test strip; and

Fig. 5 is a top view of the test strip shown in Fig. 4 after development of the test strip.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Fig. 1 illustrates a chemical test strip 10 according to the present invention. The test strip 10 is comprised of a support layer 14, a plurality of assay areas 18 and a plurality of hydrophobic zones 22. The assay areas 18 are

attached to the support strip 10 by an adhesive layer 26. The support layer 14 is comprised of a rigid chemically inert material such as a polystyrene strip having a thickness "t" of approximately 10 mil. An elongated portion of the support layer 14, which does not have any of the assay areas 18 positioned on it, functions as a handle 28. The support layer 14 also has a front side 30 and a back side 34.

The assay areas 18 are comprised of a hydrophilic material 35, such as cellulose paper. The assay areas 18 have a thickness "a" which extends from the adhesive layer 26 to an upper surface 36. In the preferred embodiment, bibulous (absorbent) filter paper, such as Ahlstrom Quantitative paper number 222, is used as the hydrophilic material 35 that forms the assay areas 18.

In Fig. 1, the front side 30 includes a first assay area 38, a second assay area 42, a third assay area 46 and a fourth assay area 50. Each assay area 18 is impregnated with a chemical mixture that is selected based on the particular assay being performed. Generally, the chemical mixture is applied to the assay area 18 in liquid form. The assay area 18 is then allowed to dry, leaving the chemical mixture absorbed in the pores of the hydrophilic material 35. The chemical mixture can be any type of chemical system capable of detecting analytes in aqueous solutions, such as chemical systems for detecting chlorine, bromine, hydrogen ion (pH), hydroxyl ion (alkalinity), copper, lead, nitrate and various substances, like calcium and magnesium, that contribute to water hardness.

In the preferred embodiment, all of the assay areas on one side of the test strip 10 contain chemical mixtures selected to detect the same analyte. For example, all of the assay areas 38, 42, 46 and 50 may contain chemicals that detect free available chlorine. However, the specific composition of the chemical mixture on a particular assay area 18 differs in some way from the chemical mixture on every other assay area 18. For example, the chemical mixture on the assay area 38 may differ from the chemical mixture on the assay area 42 in the concentration of a particular component. Similarly, the chemical mixture on the assay area 38 may contain completely different chemicals from

the chemical mixture on the assay area 50, even though both assay areas detect the same analyte (i.e. free available chlorine).

Similarly, the back side 34 includes a fifth assay area 54, a sixth assay area 58 and a seventh assay area 62. Generally, the assay areas on the back side 34 will contain chemicals selected to perform a different assay than is performed by the assay areas on the front side 30. For example, the assay areas 54, 58 and 62 may all contain chemicals that detect available bromine. However, as with the assay areas 18 on the front side 30, the specific composition of the chemical mixture on a particular assay area 18 located on the back side 34 differs in some way from the chemical mixture on every other assay area 18 located on the back side 34.

The hydrophobic zones 22 are barriers that minimize the migration of aqueous solutions between adjacent assay areas 18. In the preferred embodiment, the hydrophobic zones 22 have a thickness "d" that extends from the adhesive layer 26 up to the top surface of the hydrophobic zone 22.

Fig. 2 illustrates that each hydrophobic zone 22 comprises a channel (lane) 63 positioned between adjacent assay areas 18, such as between the assay areas 46 and 50. The channel 63 is filled (or coated) with a hydrophobic material 64, such as a hydrophobic glue or a hydrophobic polymer. A compressed paper layer 65 forms the bottom of the channel 63 and separates the adhesive layer 26 from most of the hydrophobic material 64.

In the preferred embodiment, the channel 63 is roughly trapezoidal in shape and the top edge is indented slightly from the surface 36. In other words, the thickness "d" equals the thickness "a" along the interface of the hydrophobic zones 22 with the assay areas 18, and the thickness "d" is slightly less than the thickness "a" in the middle of one of the hydrophobic zones 22.

In the preferred embodiment, the hydrophobic zones 22 are formed by embossing a plurality of the channels 63 into a continuous rectangular shaped piece of the hydrophilic material 35. The embossing process compresses the hydrophilic material 35 in the channel 63, thereby forming the paper layer 65. Each channel 63 is then filled with the hydrophobic material 64. The

hydrophobic material 64 fills the voids in the paper layer 65 so that each channel 63 is essentially an impervious barrier to the flow of aqueous solutions between adjacent assay areas 18.

In the preferred embodiment, an ethylvinylacetate glue, such as a 70:30 mixture of commercially available Elmer's ® Glue-All glue (available from Borden, Inc.) and water is used as the hydrophobic material 64. It should be appreciated that the hydrophobic material 64 must be compatible with the overall assay system. For example, ethylvinylacetate works in a chlorine/bromine assay. In other assays, materials such as acrylic latex, silicone, wax, parafin or ethylcellulose could function as the hydrophobic material 64. Similarly, in other embodiments, an empty channel between each of the assay areas 18 could function as the hydrophobic zone 22, as could a physical barrier such as a monofilament positioned between adjacent assay areas 18.

The adhesive layer 26 is positioned between the support layer 14 and the assay areas 18 and has a thickness "b". The function of the adhesive layer 26 is to firmly attach the assay areas 18 to the support layer 14. Desirable characteristics of the adhesive layer 26 include high initial tackiness (stickiness); remaining tacky in the presence of aqueous solution; and low free radical content to avoid interference with the assay. In the preferred embodiment, the adhesive layer 26 is commercially available double sided medical grade tape, such as that manufactured by the 3M Company under the part number 415.

The test strip 10 is used in the following manner: the test strip 10 is held by the handle 28 and dipped into an aqueous test solution containing the analyte. This wets the test strip with the test solution and initiates the assay. After an assay dependent amount of time (generally 1-10 seconds), the test strip 10 is withdrawn from the test solution. Each of the assay areas 18 have been formulated to undergo a physical change, such as a color change, in a certain concentration range of the analyte. Furthermore, the assay area 38 has been formulated to undergo the physical change in a lower concentration range than the assay area 42; the assay area 42 undergoes the physical change in a lower concentration range than the assay area 46; and the assay area 46 undergoes the

physical change in a lower concentration range than the assay area 50. Generally, the assay area 18 which measures the lowest concentration of analyte (i.e. assay area 38) is positioned farthest away from the handle 28 so as to avoid contamination of the higher concentration assay areas when the test strip is removed from the test solution.

Generally, the test strip 10 is read as soon as it is removed from the test solution, although prescribed amounts of time for development of the test strip 10 could be required in some assays. Reading of the test strip 10 is simplified because of the individually formulated assay areas 18. If only one of the assay areas 18 has undergone the physical change, for example the assay area 38, then the analyte concentration is very low. If several of the assay areas 18 have undergone the physical change, for example the assay areas 38, 42 and 46, then the analyte concentration is at a medium level. For example, Fig. 3 illustrates the situation where the assay areas 38, 42 and 54 have undergone a physical change (i.e. have changed color) relative to Fig. 1. Thus, in Fig. 3 the test strip 10 can be interpreted directly by observing how many of the assay areas 18 have undergone the physical change, without referring to an external scale, such as a colorimetric chart. This allows the test strip 10 to be read by an individual who has trouble distinguishing color changes.

Fig. 4 illustrates an embodiment of the test strip 10 in which printed characters have been applied to various parts of the test strip 10. For example, a plurality of numerals 70 have been printed on the assay areas 18. More specifically, the numeral "1" has been printed on the first assay area 38; the numeral "3" has been printed on the second assay area 42; the numeral "6" has been printed on the third assay area 46; and the numeral "9" has been printed on the fourth assay area 50. Additionally, the word "chlorine" has been printed on the handle 28 to identify the type of assay, and the terms "Lo" and "Hi" have been printed at positions on the test strip 10 that indicate the direction in which the assay proceeds.

The numerals 1, 3, 6 and 9 are chosen to indicate the concentration of the analyte in a particular test that the assay area has been formulated to detect.

In this example, the numerals represent the parts per million level (ppm) of free available chlorin in the test solution. It should be appreciated that the numerals 70, as well as the other printing, can be changed during the manufacturing of the test strips 10, depending on the design of the assay being conducted.

What is important about the numerals 70 is the material from which they are formed. The inside area of each numeral 70 is covered with a hydrophobic material 74. The area covered by the hydrophobic material 74 does not undergo a chemical reaction when the test strip 10 is dipped in the test solution.

Additionally, the color of the hydrophobic material 74 is chosen to match the color of the particular assay area 18 on which a particular numeral 70 is located. Thus, for example, if the chemical mixture on the assay area 38 changes from colorless to blue when exposed to an analyte, the hydrophobic material 74 used on the assay area 38 would be white (i.e. the color of the assay area 38 in the presence of unreacted dye). When the assay area 38 undergoes a reaction and changes to a blue color, the hydrophobic material 74 remains white. Thus, the numeral "1" will be visible on the assay area 38 as a white numeral highlighted by a blue background.

It should be noted that the specific color scheme used on the test strip 10 (e.g. blue and white) is not important. What is important is that the developed assay area contrast with the hydrophobic material 74, thereby allowing a reacted assay area 18 to be readily identified. In general, the numeral 70 should be less discernable in the unreacted state (perhaps nondiscernable), and very discernable after the particular assay area 18 has reacted. In the preferred embodiment, the hydrophobic material 74 is the same material as is used for the hydrophobic zones 22 (i.e. ethylvinylacetate). For example, Fig. 4 illustrates the case where the assay areas 38 and 42 have reacted, as is indicated by the fact that the numerals "1" and "3" are lighter than the rest of the surface area of the assay areas 38 and 42, and therefore contrast with the surrounding assay area surface area. In other embodiments, Acrylic latex, silicone, wax, parafin or ethylcellulose could also function as the hydrophobic material 74.

The following examples are illustrative of the present invention.

Example 1

In a representative assay, the assay areas 18 on the front side 30 are designed to measure the free available chlorine concentration in an aqueous sample such as pool or hot tub water. Free available chlorine means chlorine in an aqueous solution as hypochlorous acid (HOCl) , hypochlorite ion or, in strong acid solutions, as free chlorine. The composition of the chemicals mixtures on the assay areas 38, 42, 46 and 50 are formulated to indicate very low, low, medium and high available chlorine levels. The chemicals mixture on the assay area 38 contains 3, 5, 3′, 5′-tetramethylbenzidine (TMB) at a pH of 4.0 with a buffer concentration 0.10 molar citric acid. This buffer is used to keep the TMB at a pH of 4.0. The reagent transforms from colorless to blue in the low range of approximately 0.5 ppm of free available chlorine. The article Horseradish Peroxidase-Catalyzed Oxidation of 3, 5, 3′, 5′ Tetramethybenzidine, *The Journal of Biological Chemistry*, Vol. 257, No.7, pp. 3669-3675 (April 10, 1982), describes the chemistry involved.

The chemicals mixture on the assay area 42 contains the dye syringaldazine. In U.S. Pat. No. 4,092,115, Rupe et al. describe the use of two dyes, vanillin azine and syringaldazine in phosphate buffer. In the present invention, it has been determined that the system performs better using only syringaldazine, as opposed to the two dye system described by Rupe et al. The dye is mixed in a solvent mixture of denatured ethanol and acetonitrile. A 0.10 molar maleic acid solution at pH 6.0 is added to the solvent mixture to obtain a pH of 6.0. The dye is more stable at this pH and the maleic acid makes the dye more stable and results in less color fading than the phosphate buffer described by Rupe et al. The dye changes from a light yellow color to purple in the range of 1.5 to 2.0 ppm of free available chlorine.

The chemicals mixture on the assay area 46 is the same as on the assay area 42 except that sodium thiosulfate ($Na_2S_2O_3$) is added to the dye mixture. The sodium thiosulfate is an antioxidant that preferentially competes with the dyes for the free available chlorine. The concentration of sodium thiosulfate is

adjusted so that the dyes change from a light yellow color to purple in the range of 3 to 3.5 ppm of free available chlorine.

The chemicals mixture on the assay area 50 is the same as on the assay area 46 except that the concentration of sodium thiosulfate is adjusted so that the dyes change from a light yellow color to purple at a higher level than on assay area 46, such as in the range of 5 to 5.5 ppm of free available chlorine.

Example 2

In this assay, the assay areas 18 on the back side 34 are designed to measure bromine ion concentration. The chemical mixture on the assay area 54 is a mixture of sodium iodide (or potassium iodide), potato starch and dextran $[(C_6H_{10}O_5)_n]$, buffered to pH 4.0 with 0.10 molar citric acid. The dextran is used to fix the dyes so that they are less soluble in water. Dextran has been selected due to its similar structure to the starch. An antioxidant, such as sodium thiosulfate, is added to compete with the iodide for bromine. The antioxidant is added in sufficient quantity to cause the reagent to transform from colorless to yellow. The yellow iodide transforms into a blue color when reacted with the starch. In the preferred embodiment the assay area 54 is designed to change color at approximately one ppm of bromine which is found as Br_2O_2 or equivalent HOBr. A similar dye system is described by Vogel in *Textbook of Quantitative Chemical Analysis* (5th ed.), pages 384 - 416.

The chemical mixture on the assay area 58 is the same as on the assay area 54 except the concentration of antioxidant is adjusted so that the dye changes from colorless to blue in the second desired range. In the preferred embodiment the assay area 58 is designed to change color at about 3.5 ppm of bromine.

The chemical mixture on the assay area 62 is the same as on the assay area 58 except that the concentration of antioxidant is adjusted so that the dye changes from colorless to blue in the third desired range. In the preferred embodiment the assay area 62 is designed to change color at about 5.5 ppm of bromine.

Examples one and two illustrate that the chemistries for creating the binary results are formulated from various dye systems, which react at specific ranges, or by adding antioxidants to the solution which react with the analyte and delays the dye color change. Alternatively, a dye which is consumed by the reaction can be used as an indicator. This third system would create a product which converts from colored to clear as the assay area dye is consumed by the reaction. In all cases, the color change (known as thresholding) occurs when the dye (indicator) is consumed or bonded by the analyte. Table 1 below illustrates three possible formulations for the chemical mixtures applied to the assay areas 18.

Assay Type	Level A	Level B	Level C	Level D
1) Separate indicator dyes.	Dye 1	Dye 2	Dye 3	Dye 4
2) Dye or dye pairs with antioxidant interceptor.	Dye 1	Dye 1 or Dye 2 plus first level of antioxidant	Dye 1 or Dye 2 plus second level of antioxidant	Dye 1 or Dye 2 plus third level of antioxidant
3) Dye with extra dye to be consumed by the reaction.	Dye 1	Dye 1 plus X% Dye 1	Dye 1 plus Y% Dye 1	Dye 1 plus Z% Dye 1

Table 1 - Formulation Matrix

Referring again to Fig. 1, the method of functioning of the present invention can be described. The assay areas 18 are comprised of a hydrophilic material, such as cellulose paper, that provides a bibulous matrix of support material. The bibulous matrix includes a plurality of pores (spaces) that provide the volume to be occupied by the test reagent (i.e. the dye or dye system that is applied to the assay areas 18), and subsequently the test solution (i.e. the aqueous sample that contains the analyte) to form a microtitration.

The characteristics of the paper/matrix absorption must be consistent and reproducible. The total volum of the matrix less the cellulose/matrix material is known as the void volume (V_V). The volume occupied by the test reagent is known as the volume of reagent (V_R). The remaining volume is the sample volume (V_S) or titration volume (V_T).

When the test strip 10 is dipped in a test solution, each of the assay areas 18 are rehydrated. If the concentration of the analyte in the titration volume is less than the concentration of the titrant in the volume of reagent, then the dye in the assay area 18 does not change color. On the other hand, if the concentration of the analyte in the titration volume is greater than the concentration of the titrant in the volume of reagent, then the dye in the assay area 18 changes color. If one of the numerals 70 has been printed on the assay area 18, then the contrast between the developed assay area 18 and the undeveloped numeral 70 is readily apparent. The test strip is read by looking for the developed assay area 18 corresponding to the highest concentration of analyte. This will generally be the developed assay area 18 closest to the handle 28.

In order for the test strip 10 to function properly, the test strip 10 should not be immersed in the test solution for more than the prescribed interval (generally 1-10 seconds). A much longer immersion time will allow the void volume to be continuously replenished by additional analyte, thereby yielding an incorrect reading. Proper functioning of the test strip 10 requires that the test strip 10 be thoroughly wetted with the test solution and then allowed to develop without further exposure to the test solution.

The purpose of the hydrophobic zones 22 is to provide a barrier that prevents test solution that has been absorbed by one of the assay areas 18 to migrate to another assay area 18. Such migration would contaminate the adjoining titrations. The hydrophobic zones 22 also assist during manufacturing by separating the chemistries so that they are not mixed while being applied.

Referring again to Fig. 1, it should be noted that in another embodiment of the present invention, the test strip 10 could be comprised of a single assay

area 18 on the front side 30 and a single assay area 18 on the back side 34. For example, the test strip 10 could simply comprise the assay areas 50 and 62 so as to provide a "high" test for chlorine and bromine.

Although the present invention has been described in terms of the presently preferred embodiment, it is to be understood that such disclosure is not to be interpreted as limiting. Various alterations and modifications will no doubt become apparent to those skilled in the art after having read the above disclosure. Accordingly, it is intended that the appended claims be interpreted as covering all alterations and modifications as fall within the true spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A chemical test strip comprising:

a support layer;

a first assay area attached to a first side of the support layer and comprising a first test reagent responsive to a first concentration of a first analyte;

a second assay area attached to the first side of the support layer and comprising a second test reagent responsive to a second concentration of the first analyte; and

a first hydrophobic zone positioned between the first assay area and the second assay area.

2. The test strip of claim 1 wherein the first assay area is comprised of a material that provides a volumetric region that will support microtitration.

3. The test strip of claim 1 wherein the first assay area is comprised of a bibulous matrix having a sufficient void volume to support microtitration.

4. The test strip of claim 1 wherein the first hydrophobic zone comprises a lane coated with a hydrophobic material.

5. The test strip of claim 4 wherein the hydrophobic material is selected from the group consisting of ethylvinylacetate, acrylic latex, silicone, wax, parafin and ethylcellulose.

6. The test strip of claim 1 wherein the first test reagent comprises a first dye that changes color when reacted with the first analyte, and the second test reagent comprises a second dye that changes color when reacted with the first analyte.

7. The test strip of claim 1 wherein the first test reagent comprises a first dye that changes color when reacted with the first analyte, and the second test reagent comprises a mixture of the first dye and a competitive material that reacts with the first analyte before the first dye bonds to the first analyte.

8. The test strip of claim 6 wherein the first analyte comprises chlorine, the first dye comprises 3, 5, 3', 5'-tetramethylbenzidine and the second dye comprises syringaldazine.

9. The test strip of claim 7 wherein the first dye comprises syringaldazine and the competitive material comprises sodium thiosulfate.

10. The test strip of claim 7 wherein the first dye comprises a mixture of syringaldazine and maleic acid.

11. The test strip of claim 1 wherein the first test reagent comprises a mixture of a first dye and a first concentration of a competitive material that reacts with the first analyte before the first dye reacts with the first analyte, and the second test reagent comprises a mixture of the first dye and a second concentration of the competitive material.

12. The test strip of claim 11 wherein the first dye comprises a mixture of an iodide salt, dextran and a starch.

13. The test strip of claim 1 further comprising:

at least one character printed on the first assay area with a hydrophobic material.

14. The test strip of claim 1 further comprising:

a third assay area attached to a second side of the support layer and comprising a third test reagent responsive to a first concentration of a second analyte;

a fourth assay area attached to the second side of the support layer and comprising a fourth test reagent responsive to a second concentration of the second analyte; and

a second hydrophobic zone positioned between the third assay area and the fourth assay area.

15. The test strip of claim 14 wherein the third assay area is comprised of a material that provides a volumetric region that will support microtitration.

16. The test strip of claim 14 wherein the first hydrophobic zone comprises a lane coated with a hydrophobic material.

17. A chemical test strip comprising:

a support layer;

a first assay area attached to a first side of the support layer and comprising a first test reagent responsive to a concentration of a first analyte, the first assay area being comprised of a material that provides a volumetric region that will support microtitration; and

a second assay area attached to a second side of the support layer and comprising a second test reagent responsive to a concentration of a second analyte, the second assay area being comprised of a material that provides a volumetric region that will support microtitration.

18. A chemical indicator for bromine comprised of a mixture of an iodide salt, a starch and dextran.

19. A chemical indicator for chlorine comprised of a mixture of syringaldazine and maleic acid.

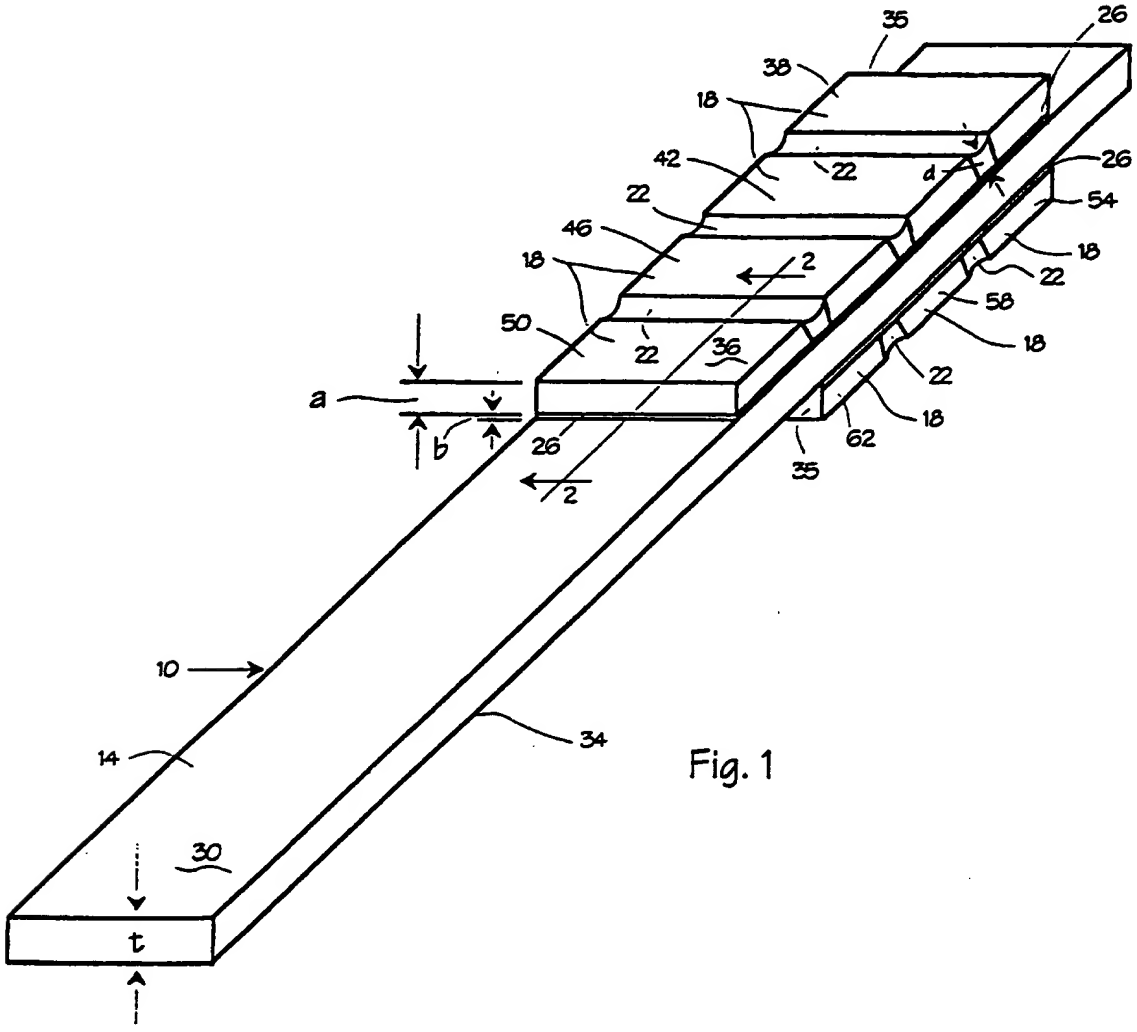

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Fig. 1

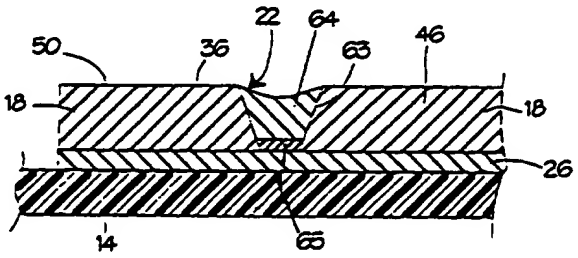
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Fig. 2

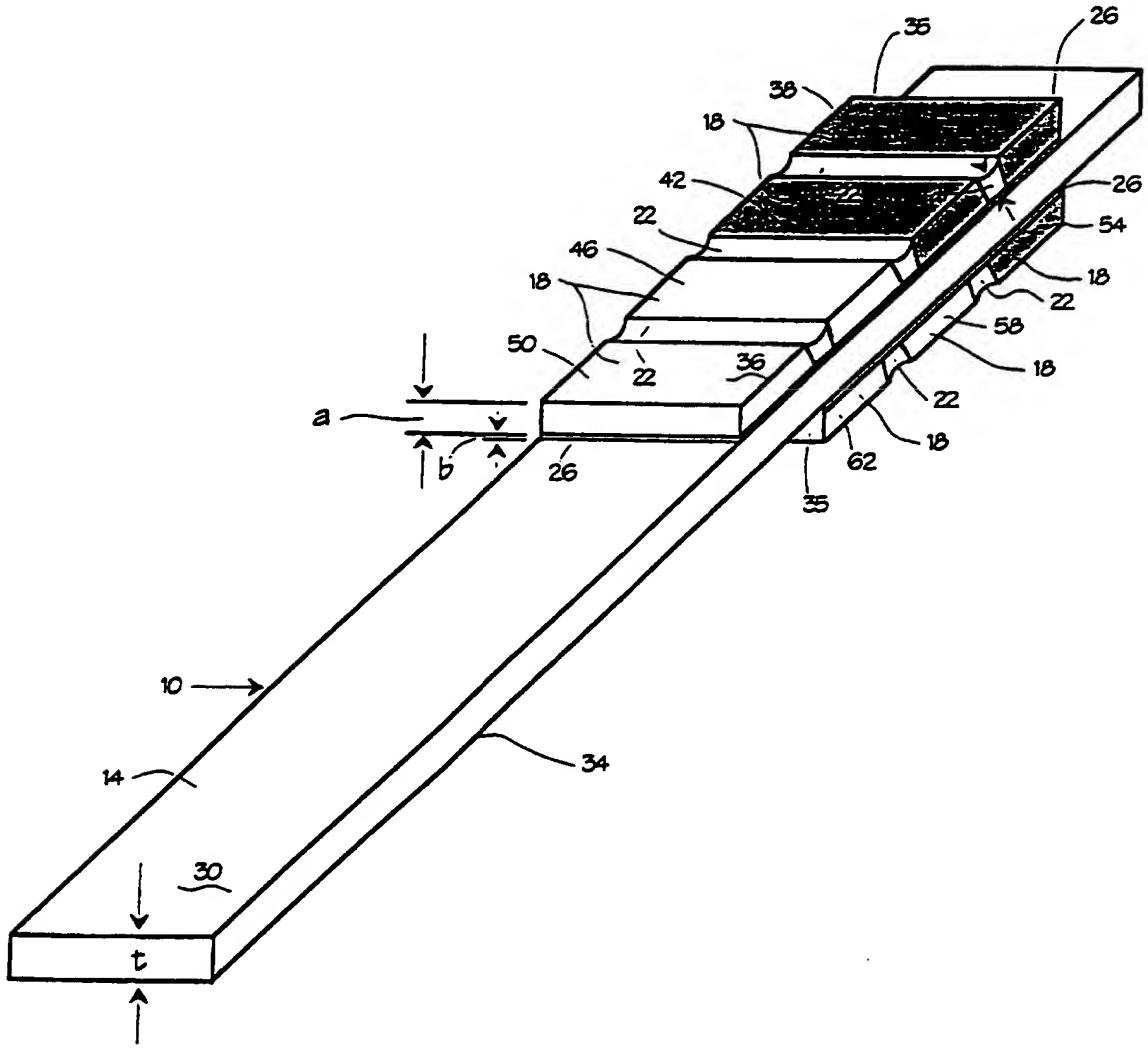
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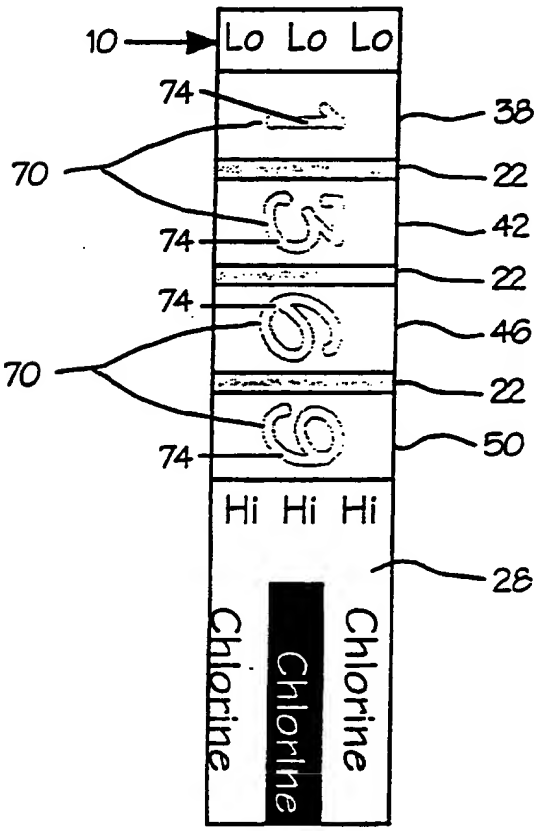
Fig. 3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Chlorine

Fig. 4

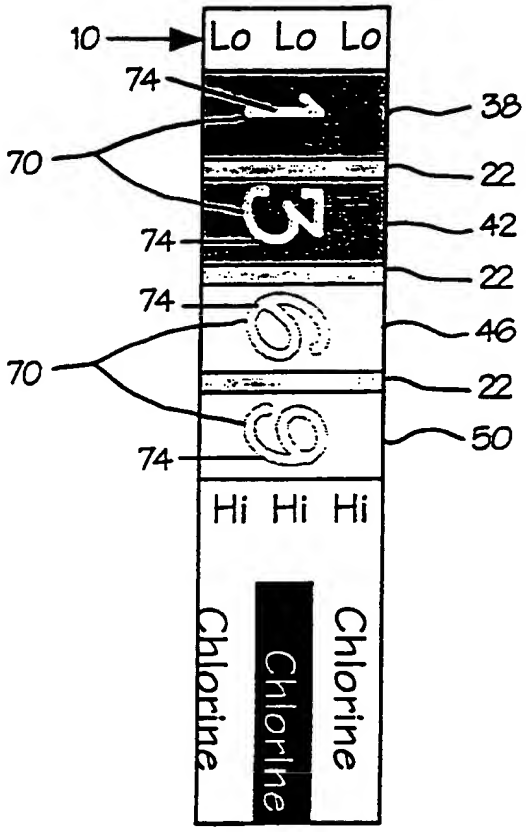
10
Lo Lo Lo
74
38
70
22
74
42
22
74
46
70
22
74
50
Hi Hi Hi
Chlorine
Chlorine
Chlorine

Fig. 5

INTERNATIONAL SEARCH REPORT

International Application No
PC ,US 96/15473

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/52 G01N31/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,82 02251 (MINNESOTA MINING & MFG) 8 July 1982 see page 4, line 18 - line 26; claims 1,5 ---	1-5
X	US,A,3 964 871 (HOCHSTRASSER HARRY) 22 June 1976 cited in the application see the whole document ---	1-5, 13-16
X	PATENT ABSTRACTS OF JAPAN vol. 012, no. 415 (P-781), 4 November 1988 & JP,A,63 151852 (TOSOH CORP), 24 June 1988, see abstract ---	18
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 1997	17 -02- 1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cartagena y Abella,P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

formation on patent family members

International Application No
PC.,US 96/15473

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